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Relationship between serum immunoglobulin G and alpha-fetoprotein levels during human pregnancy

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1 Introduction

Alterations in the complex immunoregulatory mechanisms during pregnancy must exist to explain survival to term of the histoincompatible embryo and fetus. Both the recognitive and the effector immune capabilities of the pregnant female have been examined in a number of studies and, although the results are conflicting, some evidence appears to support a suppression of these capabilities. In fact, reduction of maternal immunological responsiveness is suggested by prolonged periods of survival of skin allografts [3], by an augmented susceptibility of viral infections during pregnancy [13], by lack of cytotoxic effector cells directed specifically against paternal target cells [27], and by specific hyporesponsiveness to paternal alloantigens at the humoral level [12]. The intrinsic inhibition of maternal lymphocyte reactivity initially proposed [24] seems, on the other hand, to be giving place to the presence of inhibitory factors of lymphocyte transformation in serum of gravidas [11, 23]. Blocking antibodies [25], suppressor cells [28], and lack of antigen presentation on trophoblasts [31] appear to critically contribute to fetal non-rejection.

Among the suppressor factors present in pregnancy serum, α-fetoprotein (AFP) is one of the most investigated. Although high levels of AFP during pregnancy indicate an essential role, nothing conclusive is known about its function. According to some investigators, AFP is immunosuppressive [14, 17, 18], but others have not found any suppression in in vitro systems and have even noted a stimulatory action for AFP [8, 15].

We have already demonstrated that PHA lymphocyte proliferation, the simplest and most reproductible in vitro correlate of cell-mediated immunity [21], is not influenced by the levels of AFP encountered in pregnancy serum [23], in cord serum [32] and also in amniotic fluid [33]. Altered concentrations of immunoglobulins during pregnancy have been described and the findings of some investigators suggest that AFP may act on humoral immunity [4, 5, 17, 20, 29, 30]. The present study was designed to firstly compare the levels of the immunoglobulins G, M and A in sera of women at various stages of gestation and in sera of age matched non-pregnant females. Secondly, the levels of the immunoglobulins were correlated with the levels of AFP in gravidas' sera as an attempt to examine a possible action of AFP on humoral immunity.

2 Material and methods

2.1 Serum

Peripheral blood samples were obtained from randomly selected pregnant volunteers at various stages of gestation (11 – 14 weeks, 32 – 34

weeks and 38–40 weeks), attending the Antenatal Clinic of Newcastle General Hospital, and from non-pregnant healthy female volunteers among the nursing staff of the same hospital during 12 weeks in 1982. Control subjects (non-pregnant women) were matched for age. Gestational age was calculated from the date of last menstrual period and confirmed by clinical examination. Patients were excluded from the study for the following reasons: uncertain gestation age, manifested infection, history of allergy, diabetes, hypertension or other medical problems, symptoms or signs of threatened abortion, fetal death, suspected twins and previous birth of a child with neural tube defect. The serum was separated from the cells by centrifugation and kept frozen at −20 °C until tested.

2.2 α-fetoprotein assay

Estimation of AFP was performed using the radioimmunoassay (RIA, Gnost Kit of Hoechst Behringwerke). Duplicate estimations were made for each specimen. Within duplicates, the mean coefficient of variation was 4.21%. If within duplicates this coefficient was higher than 10% for a given sample, the estimation was repeated. Results are expressed as nanograms per milliliter.

2.3 Immunoglobulins assay

Immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin A (IgA) were determined by single radial immunodiffusion using Tri-Partigen plates of Hoechst Behringwerke. The mean coefficient of variation within duplicates for IgG, IgM and IgA were respectively 3.54%, 2.63% and 2.86%. Results are expressed as milligrams per cent.

2.4 Statistics

The statistical comparisons utilized were analysis of variance (F, Duncan New Multiple Range Test), Pearson linear and partial correlations. The raw data were applied wherever possible. In some cases a logarithmic transformation was necessary. Values of $P < 0.05$ were considered significant.

3 Results

3.1 Serum immunoglobulins and AFP levels during normal pregnancy

The mean concentrations of serum immunoglobulins G, M and A at 11–14, 32–34 and 38–40 weeks of gestation and those of non-pregnant controls, as well as AFP mean levels in pregnancy serum, are given in table I. The standard deviation and number of samples in each group are also given. Mean serum AFP levels are not presented in controls, since they were below the sensitivity of the RIA Kit employed in this study. All values were less than 10 ng/ml. The concentrations of IgG are decreased throughout gestation; whereas, IgM levels are diminished at around the beginning of the third trimester. A gradual increase in AFP concentrations as pregnancy advances was also found.

3.2 Relationship between AFP and immunoglobulins levels in pregnancy serum

Table II demonstrates the zero-order Pearson linear coefficients of correlation calculated between AFP and IgG, IgM and IgA levels in pregnancy serum, as well as the number of variable pairs and probabilities. The table further presents the partial correlation coefficiency values, keeping gestational age constant. It can be seen from the coefficiency values that AFP correlates negatively with all immunoglobulins, but significance was demonstrated only with IgG. When gestational age was fixed, the correlation (partial coefficients) remained significant for IgG.

Correlation between AFP and IgG levels within each gestational age was then determined to establish whether there is a certain period in which the association is stronger. In this case, a logarithmic transformation was necessary for AFP values, since they were somewhat skewed to the right. These coefficients at 11–14, 32–34 and 30–40 weeks of gestation appear in table III together with the number of variable pairs and probabilities. All coefficients were significant and no relevant difference between probabilities was observed.

Table I. Serum immunoglobulins and α-fetoprotein levels (mean ± S. D.) in pregnancy.

	IgA (mg%)	IgM (mg%)	IgG (mg%)	Prob[A]	AFP (ng/ml)
Controls	180 ± 69 (n = 57)	172 ± 67 (n = 57)	1430 ± 313 (n = 57)		
Gestation					
11–14 wks	170 ± 52 (n = 26)	167 ± 68 (n = 26)	1158 ± 275 (n = 26)	*	32 ± 22 (n = 26)
32–34 wks	179 ± 73 (n = 37)	147 ± 58 (n = 37)	1020 ± 279 (n = 37)	*	220 ± 110 (n = 37)
30–40 wks	181 ± 58 (n = 38)	179 ± 70 (n = 38)	967 ± 218 (n = 38)	*	228 ± 81 (n = 38)
F Prob	> 0.05	> 0.05	< 0.05		

[A] Probabilities of the mean immunoglobulin differences between controls and samples from pregnant females at the various gestational ages taken separately calculated by the Duncan multiple range test.
* Significant

Table II. Relationship between α-fetoprotein and immunoglobulin G, M and A levels in pregnancy sera.

	Pearson coefficient r	No. of samples	F Probability	Partial coefficient with gestation age fixed	No. of samples	F Probability
AFP-IgG	−0.261	101	0.004	−0.181	100	0.035
AFP-IgM	−0.156	101	0.058	−0.114	100	0.127
AFP-IgA	−0.037	101	0.354	−0.052	100	0.300

Regression analyses were performed on individual paired samples.

Table III. Relationship between α-fetoprotein and immunoglobulin G serum levels at three different stages of pregnancy.

Weeks of gestation	Pearson coefficient r	No. of samples	F Probability	Partial coefficient with gestation age fixed	No. of samples	F Probability
11–14	−0.435	26	0.019	−0.485	25	0.011
32–34	−0.335	37	0.018	−0.338	36	0.019
38–40	−0.366	38	0.010	−0.352	37	0.014

4 Discussion

Several substances encountered in high concentration in pregnancy sera have been shown to possess immunosuppressive properties. Hormones such as human chorionic gonadotrophin [1], human chorionic somatomammotrophin [9], progesterone [16] and human placental lactogen [7] and proteins raised during pregnancy such as pregnancy associated α-2-globulin [10], pregnancy specific β-1-glycoprotein [7] and α-fetoprotein [14, 17, 18] are among them. Specifically regarding AFP, the results are contradictory [8, 15]. Our previous studies strongly suggest that AFP is not principally involved with the in vitro depression of cell mediated immunity caused by sera of gravidas and newborns

respectively [23, 32] nor by human amniotic fluid [33]. This study firstly approached humoral immunity of gravidas by studying the levels of immunoglobulins in pregnancy sera. We found consistently diminished IgG serum levels throughout gestation. Data published in the literature differ regarding the levels of the various immunoglobulins during pregnancy, but it has been generally found that IgG concentrations are diminished during gestation in accordance with our results [4, 22, 29, 30]. The present investigation also demonstrated a significant negative correlation between AFP and IgG levels. To our knowledge nothing has been so far published on this matter nevertheless, some investigators tried to correlate immunoglobulins with AFP levels in cord serum [6, 26]. CEDERQVIST et al. [6] found no association, whereas SCHUURMAN et al. [26] encountered a relationship between low cord IgA concentrations and high AFP levels at term. The relationship encountered in the present work is difficult to interpret since it may represent a false association. In view of various studies indicating that AFP acts at the level of the cellular interactions which lead to IgG production [2, 17, 19, 20], it is tempting to speculate that AFP may be involved in the determination of the final concentrations of IgG in maternal circulation. OGRA et al. (1974) reported that amniotic fluid AFP suppresses antibody synthesis when administered in vivo [20]. MURGITA and TOMASI demonstrated that pure AFP inhibits the in vitro primary IgM and secondary IgA and IgG antibody response in mice [17]. MURGITA et al. [19] in turn proved that murine AFP induces the formation of a suppressor T cell population, and this was also demonstrated in humans by ALPERT's investigations [2]. Since it is established that these suppressor cells are able to inhibit helper T cells ultimately linked to antibody production after proper stimulation of B cells, an immunosuppressive effect of α-fetoprotein during pregnancy limited to a certain population of lymphocytes responsible for a monoclonal suppression of cells involved in IgG synthesis is possible. If this is correct, the presently accepted lack of influence of AFP on cellular immunity may be due to the fact that this subpopulation of cells is too small to be detected in the cellular growth response experiments which were used as a screen to identify alterations of cell mediated immunity.

Summary

The immunoregulatory processes operating during pregnancy that allow the survival of the semiallogeneic conceptus are at present far from understood. α-fetoprotein (AFP) is a biological component of the body produced in high amounts during pregnancy mainly by the fetal liver, and in certain clinical pathological states. The biological function of AFP is still unknown, but some investigators postulate an immunosuppressive role for the protein during pregnancy. In this study, serum immunoglobulin G, M and A levels of 101 gravidas at different stages of gestation (26 from 11–14 weeks, 37 from 32–34 weeks and finally 38 from 38–40 weeks) were determined and compared to 57 age matched non-pregnant females. Before being included in the study, patients were checked for various conditions which potentially alter immunoglobulin and AFP serum levels. Maternal serum samples showed a significant decreased concentration of immunoglobulin G (IgG) as compared to non-pregnancy serum samples (table I). AFP levels were also quantitated in the pregnancy sera and correlated with immunoglobulins levels. A strongly negative correlation between AFP and IgG was found throughout gestation (table II). When gestational age was fixed and the partial coefficients of correlation calculated, the inverse correlation persisted. In addition, the relationship between AFP and IgG at the various periods of gestation (11–14 weeks, 32–34 weeks and 38–40 weeks), calculated in order to detect where the association was stronger, revealed significant and uniform negative correlations (table III). These results do not prove a true association. In view of other reports indicating that AFP can block cellular interactions which lead to IgG production in vitro and in vivo, they may suggest a possible immunoregulatory function for AFP at the humoral level during pregnancy.

Keywords: Alpha-fetoprotein, immunoglobulins, pregnancy serum.

Zusammenfassung

Beziehung zwischen IgG- und AFP-Spiegel während der Schwangerschaft

Die immunregulatorischen Vorgänge, die sich während der Schwangerschaft entwickeln und ein Überleben des semiallogenen Embryos ermöglichen, sind zur Zeit noch nicht genügend aufgeklärt. Alpha-Fetoprotein (AFP) ist ein biologischer Bestandteil des Blutes, der während der Schwangerschaft hauptsächlich in der fetalen Leber und bei bestimmten Krankheitsbildern in großen Mengen produziert wird. Die biologische Funktion ist noch unbekannt, doch einige Untersuchungen weisen auf die Bedeutung des Proteins bei der Immunsuppression während der Schwangerschaft hin. In dieser Studie wurden die Serumspiegel der Immunglobuline G, M und A bei 101 schwangeren Frauen mit unterschiedlichem Gestationsalter (26 Frauen in der 11.–14. SSW, 37 in der 32.–34. SSW und 38 in der 38.–40. SSW) bestimmt und mit 57 gleichaltrigen, nichtschwangeren Frauen verglichen. Vor der Messung wurden die Frauen untersucht; diejenigen, bei den Faktoren vorlagen, die potentiell die Immunglobulin- und AFP-Spiegel verändern, wurden von der Studie ausgeschlossen. Bei Schwangeren war die IgG-Konzentration im Serum bedeutend geringer als bei Nichtschwangeren (Tab. I). Ebenso wurden die AFP-Spiegel bei den Schwangeren bestimmt und mit den Immunglobulinspiegeln verglichen. Während der gesamten Schwangerschaft ergab sich eine stark negative Korrelation zwischen AFP und IgG (Tab. II). Bei gleichem Schwangerschaftsalter und Berechnung der partiellen Korrelationskoeffizienten blieb es bei einer umgekehrten Korrelation. Darüber hinaus wollten wir feststellen, ob es zwischen den einzelnen Schwangerschaftsabschnitten unterschiedlich starke Korrelationen gab. Es zeigten sich jedoch in allen Phasen (11.–14. SSW, 32.–34. SSW und 38.–40. SSW) signifikante, uniforme negative Korrelationen (Tab. III). Diese Ergebnisse sind nicht beweisend für einen echten Zusammenhang. Es gibt jedoch andere Studien, die zeigen konnten, daß AFP zelluläre Interaktionen, die zur IgG-Produktion führen, in vitro und in vivo blockieren kann. Auf diesem Hintergrund wäre eine immunregulatorische Funktion des AFP während der Schwangerschaft durchaus vorstellbar.

Schlüsselwörter: Alpha-Fetoprotein, Immunglobuline, Schwangerschaftsserum.

Résumé

Relations entre les taux d'immunoglobuline G et d'alpha-fœtoprotéine dans le sérum de femmes enceintes

Les processus immunorégulateurs qui agissent pendant la grossesse en permettant la survie du fœtus semiallogénique ne sont pas connus à l'heure actuelle. L'alpha-fœtoprotéine (AFP) est un composant biologique du corps produit en grande quantité pendant la grossesse, surtout par le foie du fœtus, mais aussi dans certaines pathologies cliniques. La fonction biologique de l'AFP n'est pas encore connue, cependant les chercheurs postulent une fonction immunosupressive pour cette protéine pendant la grossesse. Nons avons étudié les taux des immunoglobulines G, M et A dans le sérum de 101 femmes enceintes à differentes périodes de la grossesse (26 de 11 à 14 semaines, 37 de 32 à 34 semaines et 38 de 38 à 40 semaines). Ces taux ont été comparés à ceux de 57 femmes non enceintes de même âge. Avant d'être étudiées, les patientes étaient testées pour les differentes conditions qui pourraient modifier les niveaux d'immunoglobulines et d'AFP dans le sérum. Les echantillons de sérum maternel étudiés ont presenté une diminution significative de la concentration d'immunoglobuline G (IgG) comparé à celle du sérum de femmes non enceintes (tableau I). Il a été trouvé une corrélation négative très grande entre l'AFP et l'Ig G pendant la grossesse (tableau II). Quand la période de grossesse était fixée et les coefficientes partiels de corrélation étaient calculés, il persistait la corrélation inverse. De plus, la relation entre l'AFP et l'IgG à differentes périodes de grossesse (11 à 14 semaines, 32 à 34 semaines et 38 à 40 semaines) a été calculée pour détecter l'association la plus intense. Cette relation a montré des corrélations négatives significatives et uniformes (tableau III). Ces résultats ne prouvent pas une vraie association. Cependant ils peuvent suggérer une fonction immunorégulatrice probable de l'AFP au niveau humoral pendant la gestation. Ces résultats sont en accord avec d'autres montrant le blocage des interactions cellulaires par l'AFP induisant la production d'IgG in vitro et in vivo.

Mots-clés: Alpha-fœtoprotéine, immunoglobulines, sérum des femmes enceintes.

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